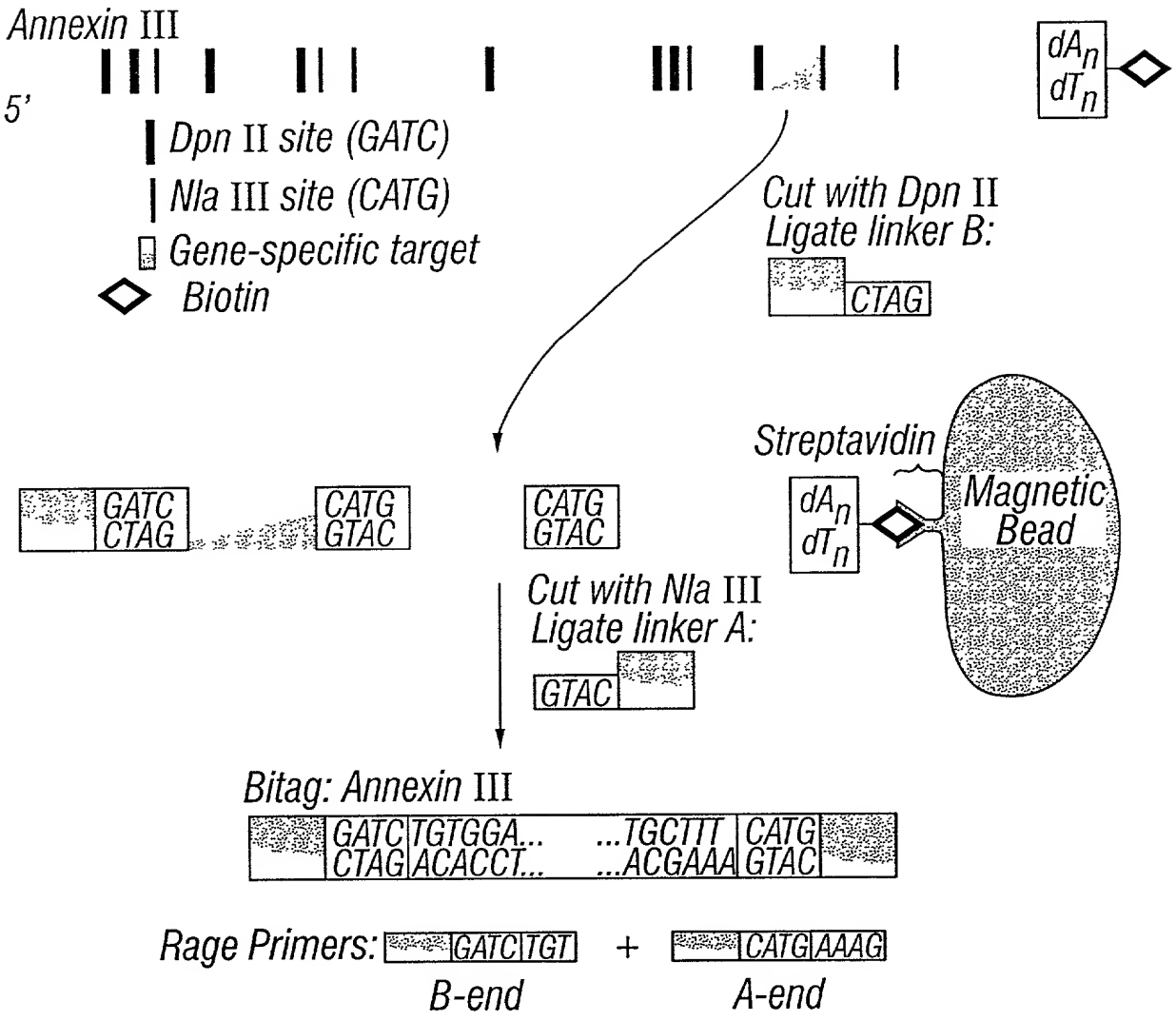
Annexin III
5'
Dpn II site (GATC)
Nla III site (CATG)
Gene-specific target
Biotin
dA n
dT n
Cut with Dpn II
Ligate linker B:
CTAG
Streptavidin
Magnetic Bead
GATC
CTAG
CATG
GTAC
CATG
GTAC
Cut with Nla III
Ligate linker A:
GTAC
Bitag: Annexin III
GATC TGTGGA... ...TGCTTT CATG
CTAG ACACCT... ...ACGAAA GTAC
Rage Primers: GATC TGT + CATG AAAG
B-end
A-end

FIG. 1

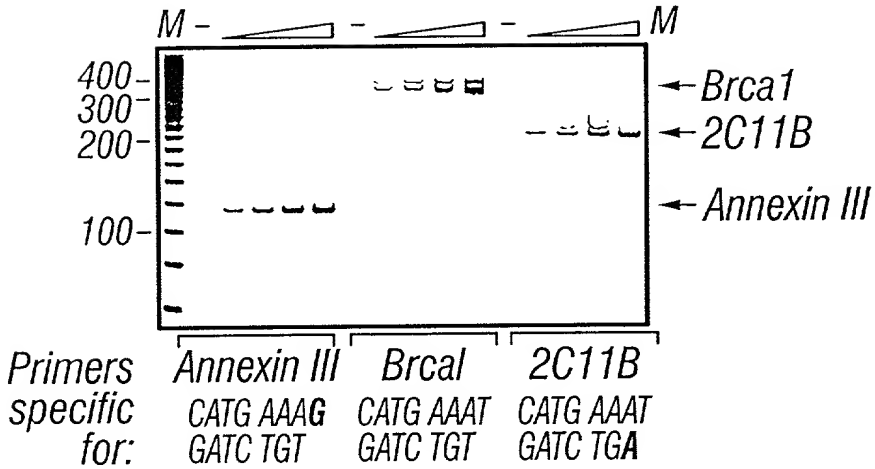
M
M
400
300
200
100
Brca1
2C11B
Annexin III
Primers specific for:
Annexin III
CATG AAAG
GATC TGT
Brcal
CATG AAAT
GATC TGT
2C11B
CATG AAAT
GATC TGA

FIG. 2A

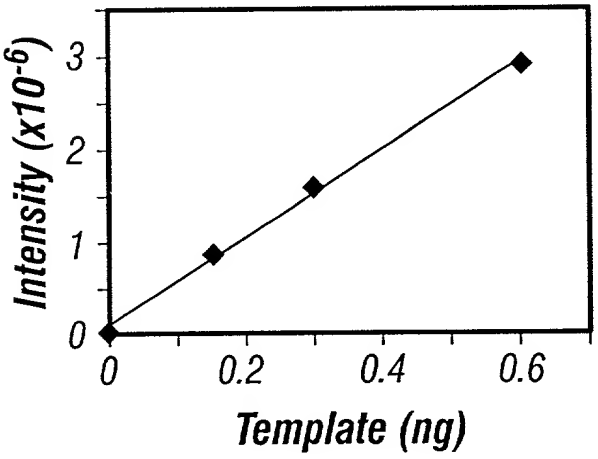
Intensity (x10-6)
3
2
1
0
0
0.2
0.4
0.6
Template (ng)

FIG. 2B

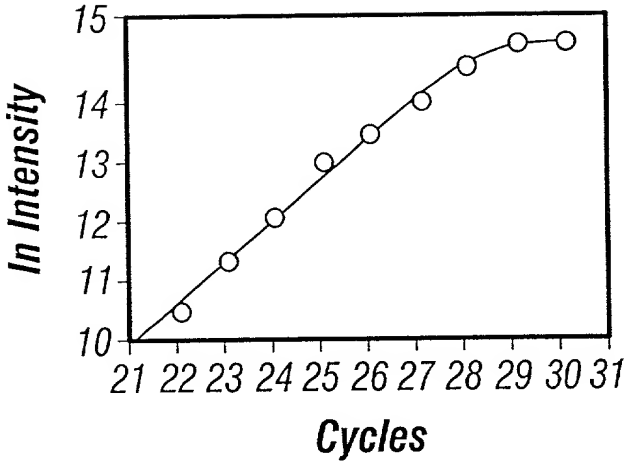
15
14
13
12
11
10
ln Intensity
21 22 23 24 25 26 27 28 29 30 31
Cycles

FIG. 2C

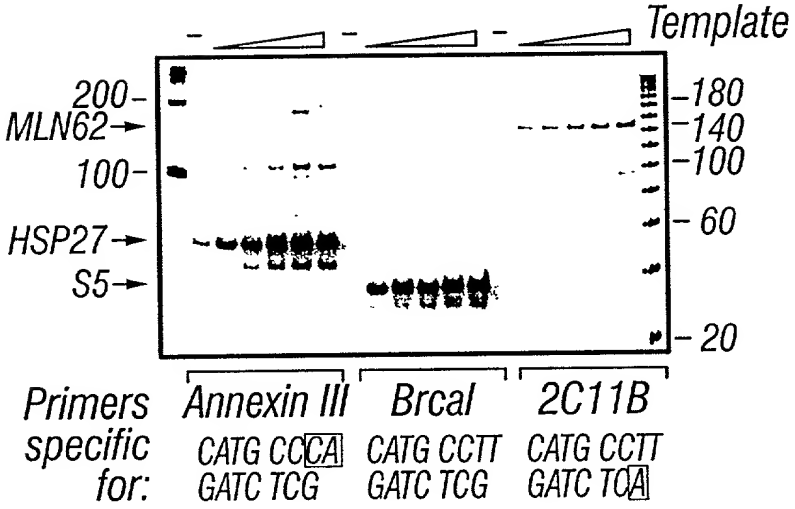
Template
200-
MLN62→
100-
HSP27→
S5→
-180
-140
-100
- 60
- 20
Primers specific for:
Annexin III
CATG CCCA
GATC TCG
Brcal
CATG CCTT
GATC TCG
2C11B
CATG CCTT
GATC TCA

FIG. 2D

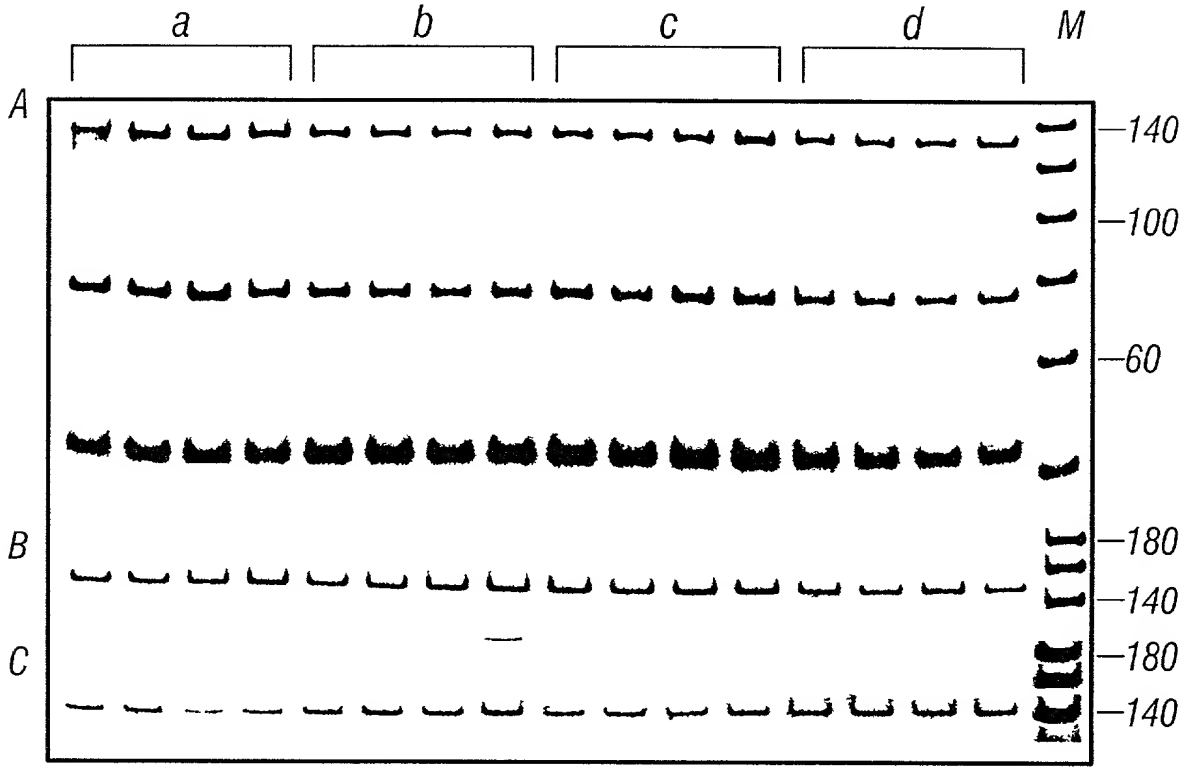

FIG. 3

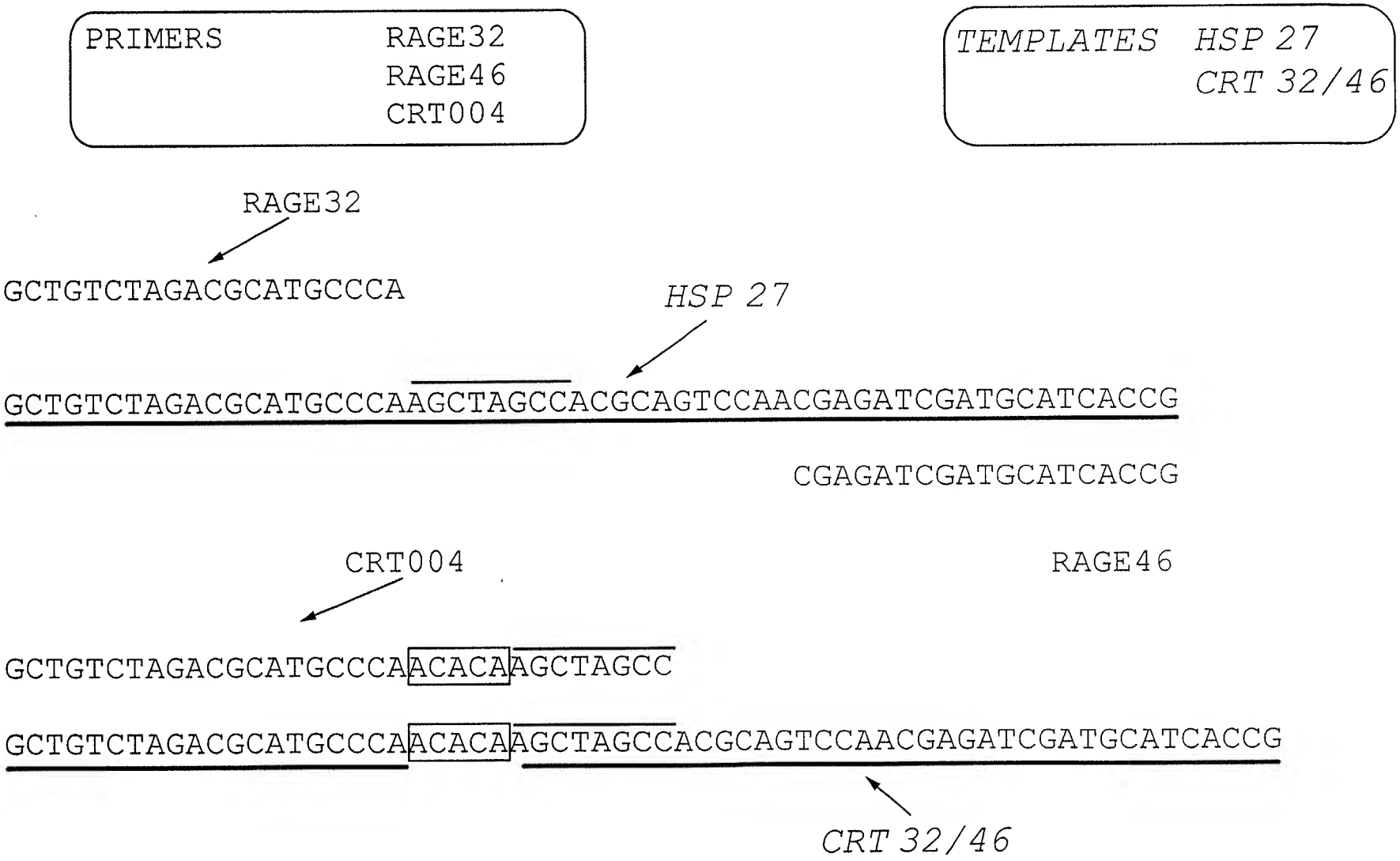
PRIMERS
RAGE32
RAGE46
CRT004
TEMPLATES
HSP 27
CRT 32/46
RAGE32
GCTGTCTAGACGCATGCCCA
HSP 27
GCTGTCTAGACGCATGCCCAAGCTAGCCACGCAGTCCAACGAGATCGATGCATCACCG
CGAGATCGATGCATCACCG
CRT004
RAGE46
GCTGTCTAGACGCATGCCCAACACAAGCTAGCC
GCTGTCTAGACGCATGCCCAACACAAGCTAGCCACGCAGTCCAACGAGATCGATGCATCACCG
CRT 32/46

FIG. 4

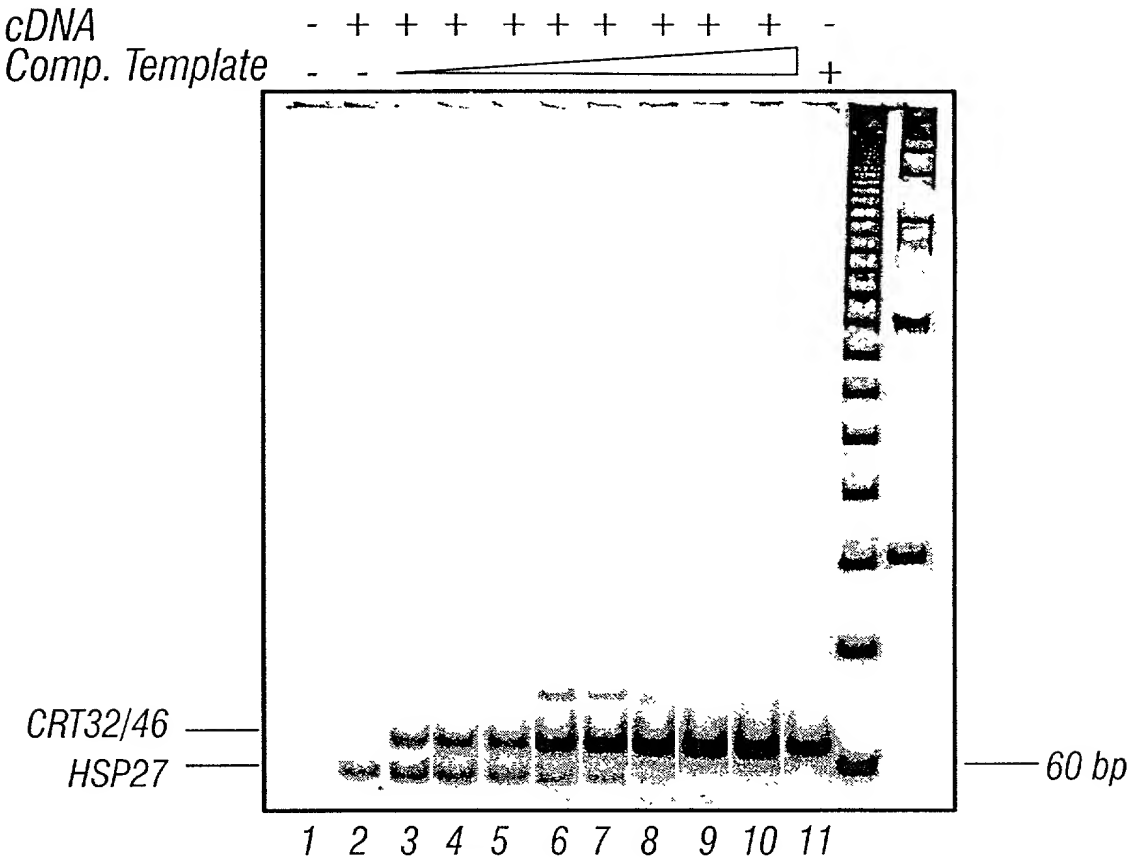
cDNA
- + + + + + + + + + -
Comp. Template
- - +
CRT32/46
HSP27
60 bp
1 2 3 4 5 6 7 8 9 10 11

FIG. 5

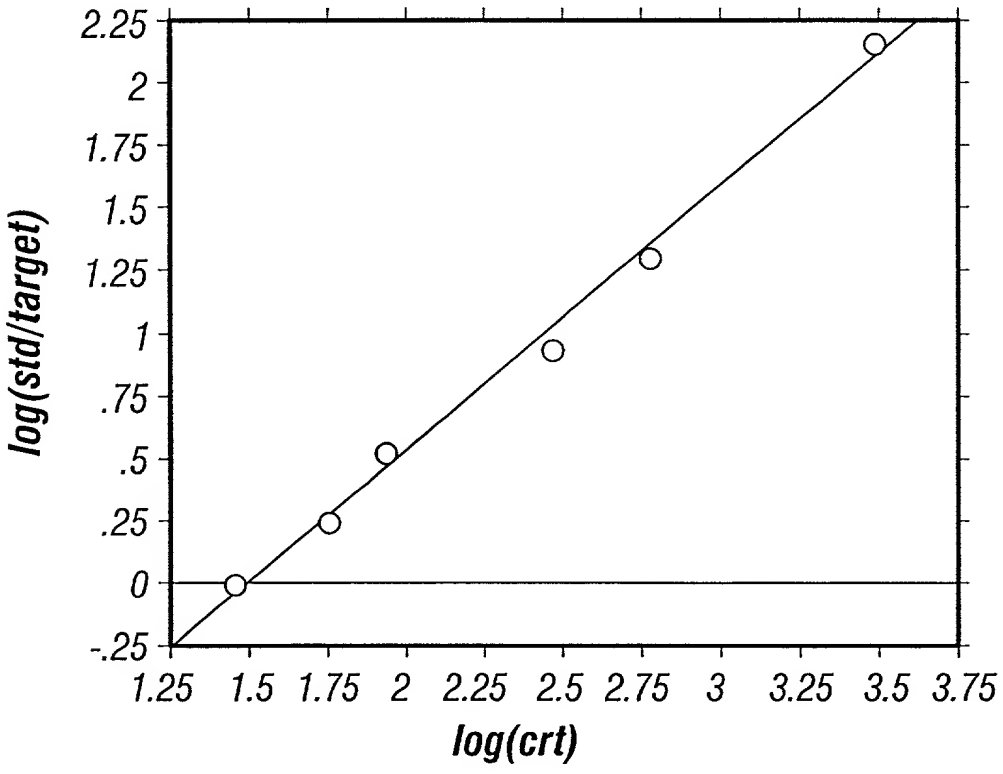
log(std/target)
2.25
2
1.75
1.5
1.25
1
.75
.5
.25
0
-.25
1.25 1.5 1.75 2 2.25 2.5 2.75 3 3.25 3.5 3.75
log(crt)

FIG. 6

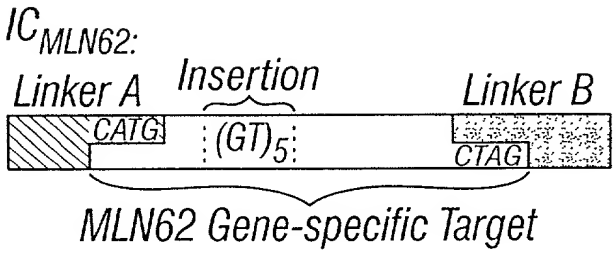

FIG. 7A

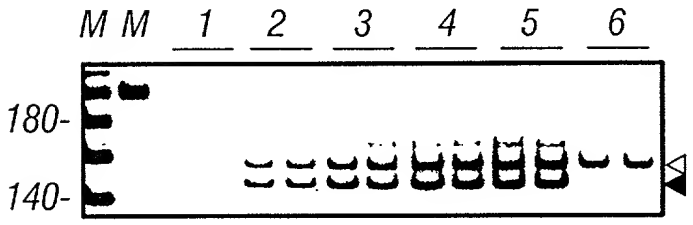

FIG. 7B

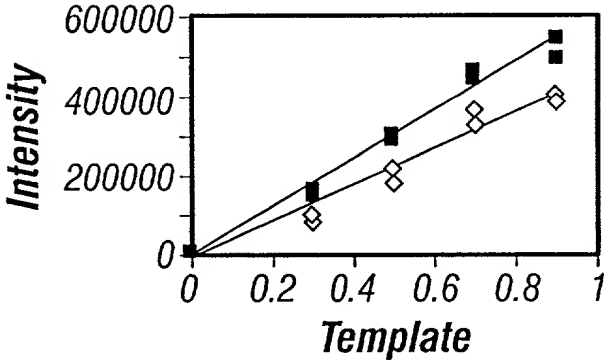

FIG. 7C

1751 TAGGGCAGAC **ATGCCTT**GGT GCCGGTCACA CTCTACACGG ACTGAGGTGC

1801 CTGCTCAGGT GCTATGTCCC AAGAGCCATA AGGGGGTGGG AATTGGGGAG

1851 GGAGAAAGGG TAGTTCAAAG AGTCTGTCT**T GAGATC**TGAT TTTTTCCCCC

1901 TTTACCTAGC TGTGCCCCCT CTGGTTATTT ATTTCCTTAG TGCCAGGAGG

1951 GCACAGCAGG GGAGCCCTGA TTTTTAATAA ATCCGGAATT GTATTTATT

FIG. 8

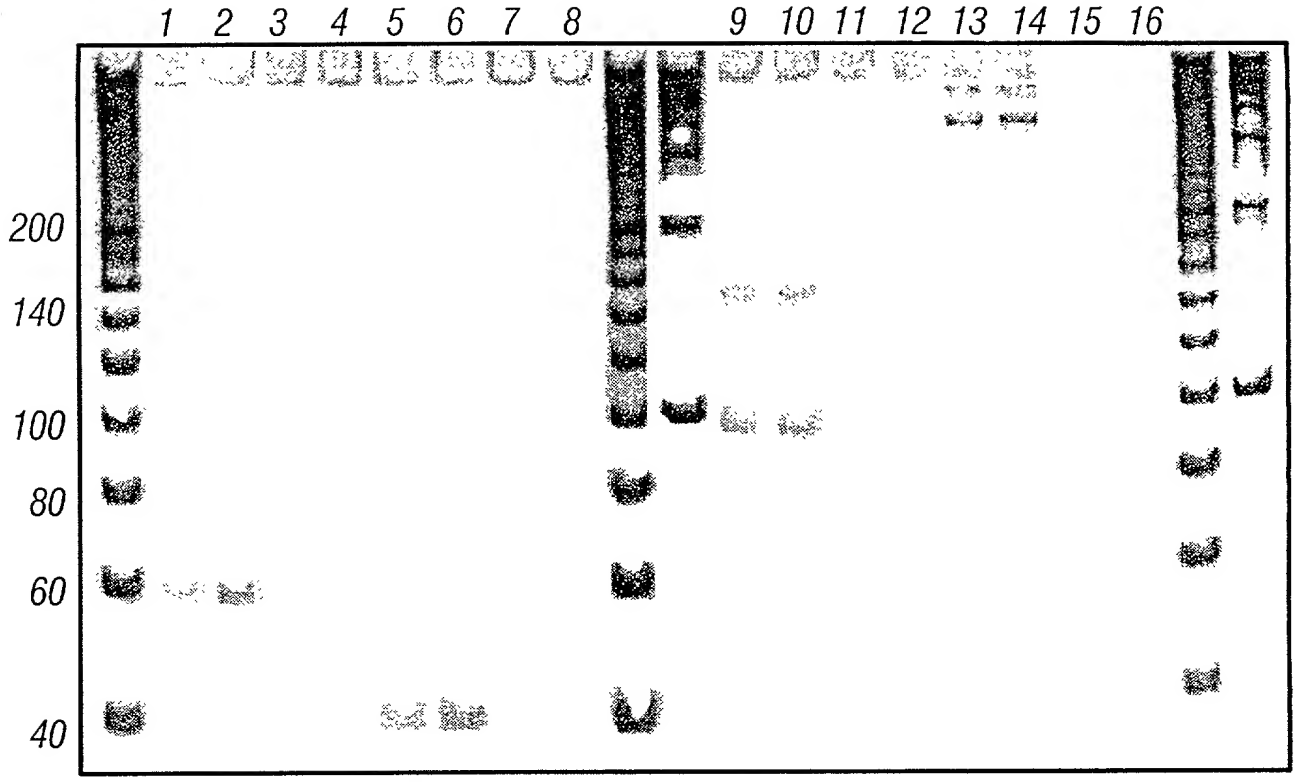
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
200
140
100
80
60
40

FIG. 9

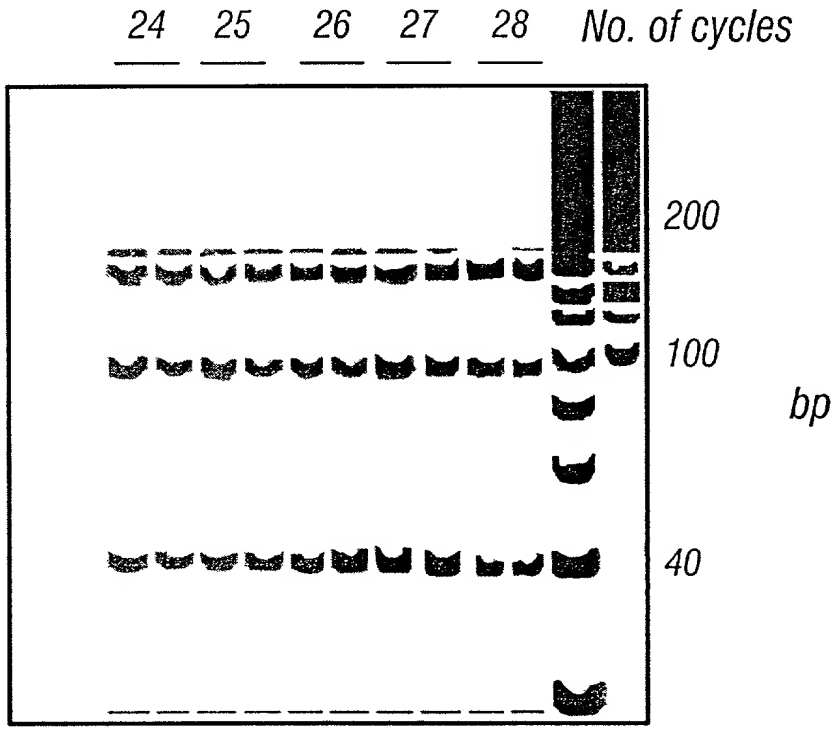
24 25 26 27 28 No. of cycles
200
100
40
bp

FIG. 10

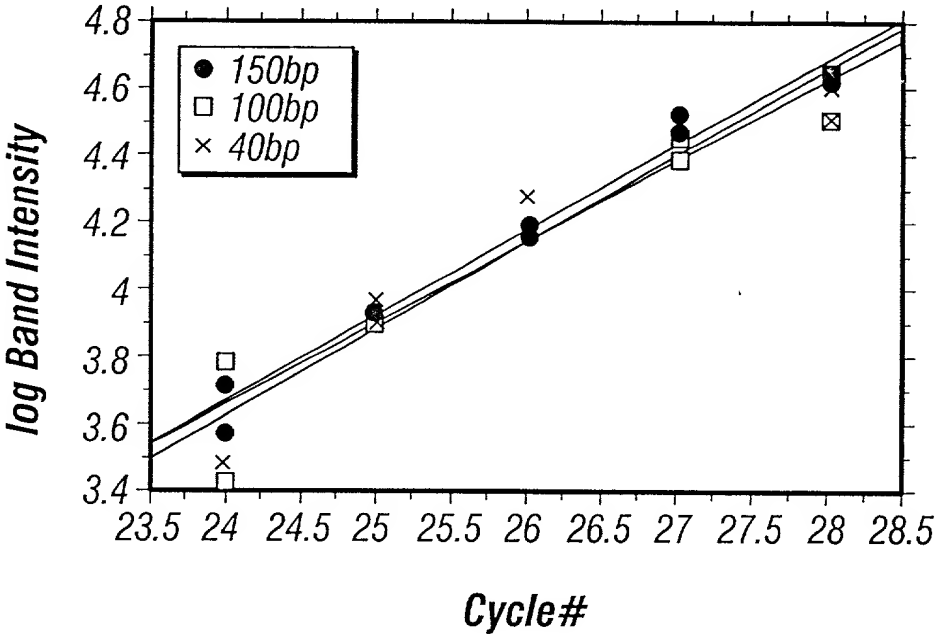
4.8
4.6
4.4
4.2
4
3.8
3.6
3.4
log Band Intensity
150bp
100bp
40bp
23.5
24
24.5
25
25.5
26
26.5
27
27.5
28
28.5
Cycle#

FIG. 11

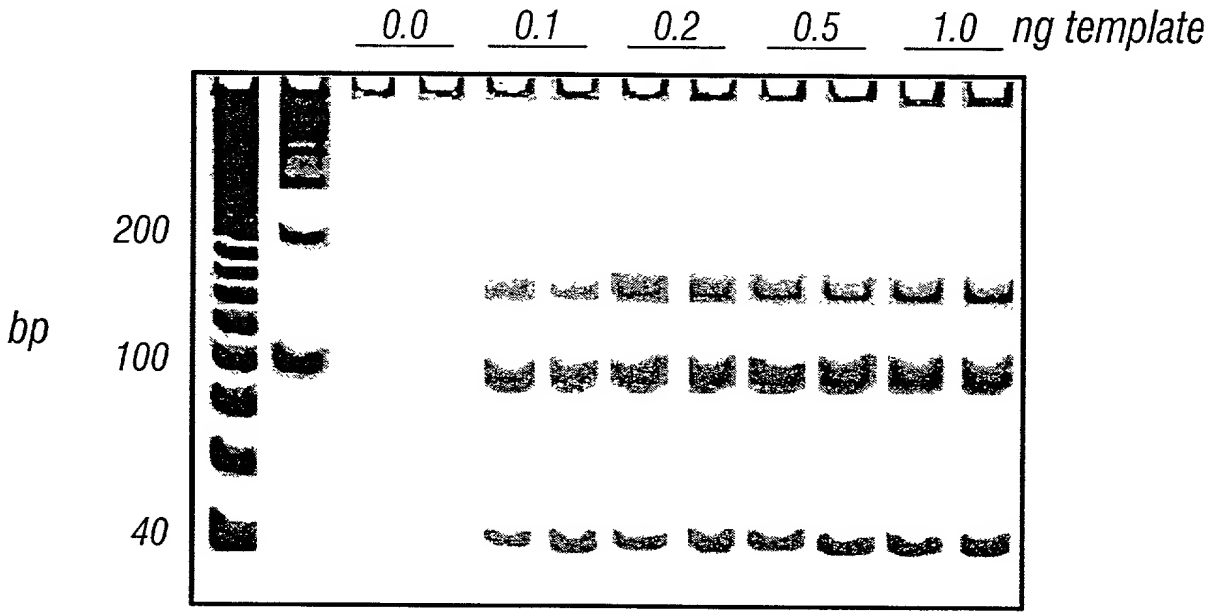
0.0
0.1
0.2
0.5
1.0
ng template
bp
200
100
40

FIG. 12

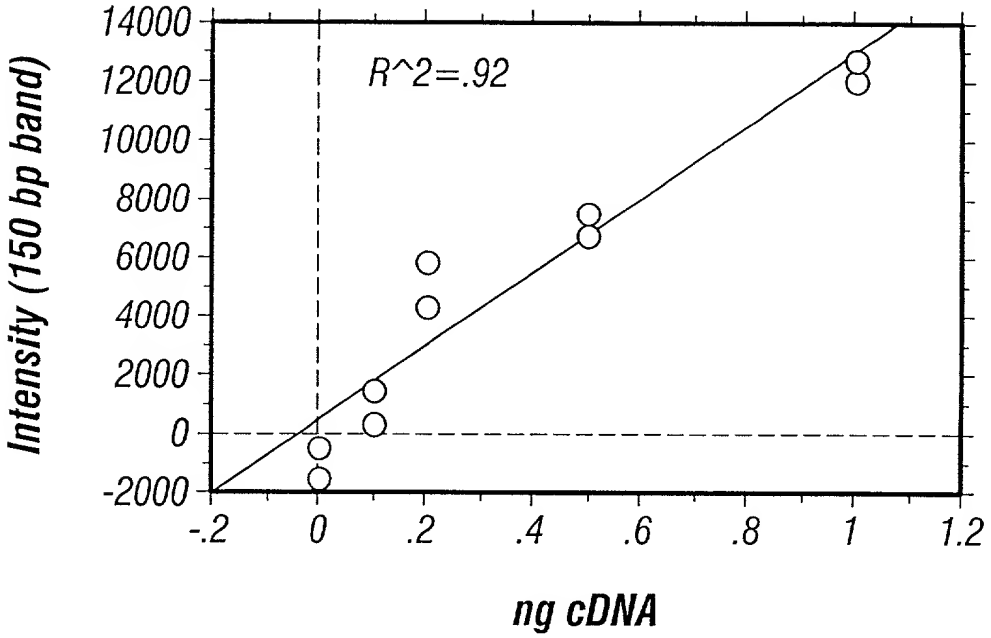
Intensity (150 bp band)
14000
12000
10000
8000
6000
4000
2000
0
-2000
R^2=.92
-.2
0
.2
.4
.6
.8
1
1.2
ng cDNA

FIG. 13

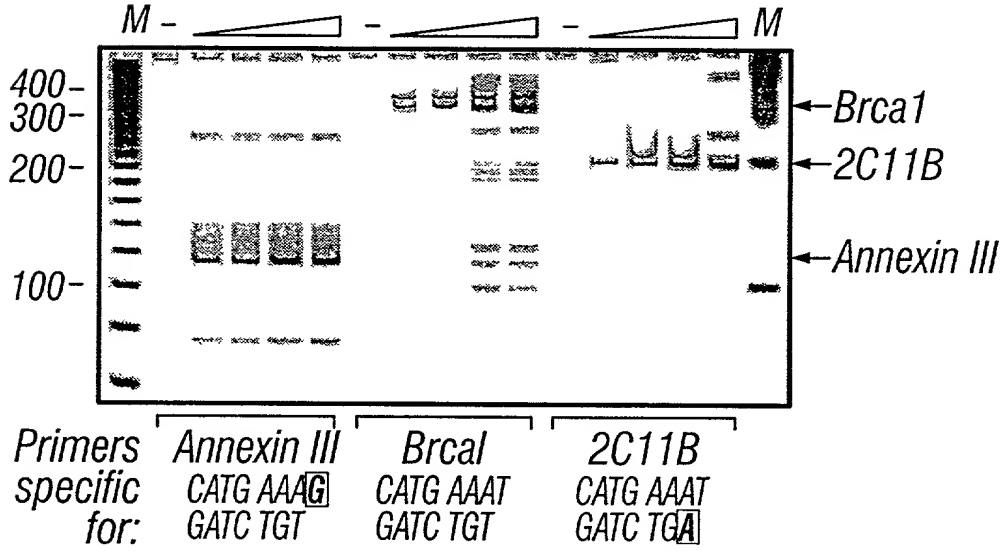
M
M
400
300
200
100
Brca1
2C11B
Annexin III
Primers specific for:
Annexin III
CATG AAAG
GATC TGT
Brcal
CATG AAAT
GATC TGT
2C11B
CATG AAAT
GATC TGA

FIG. 14

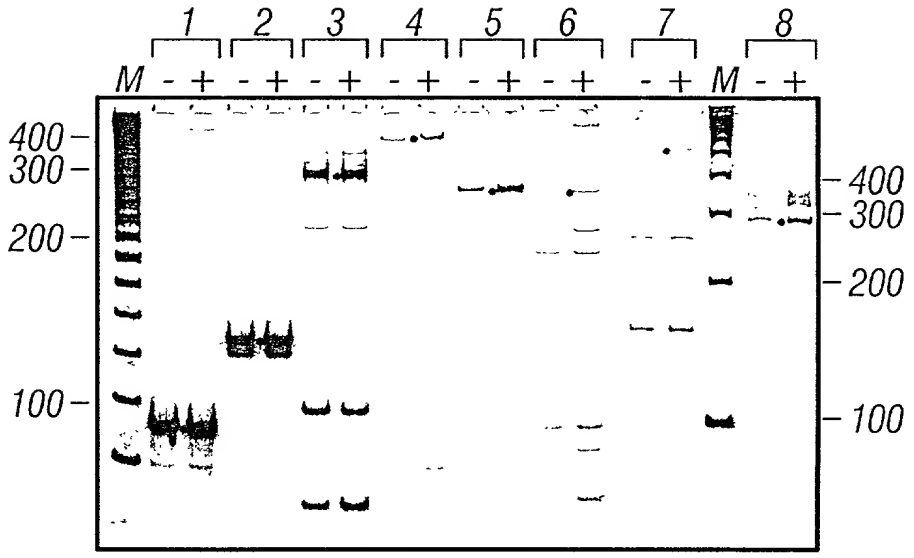

FIG. 15

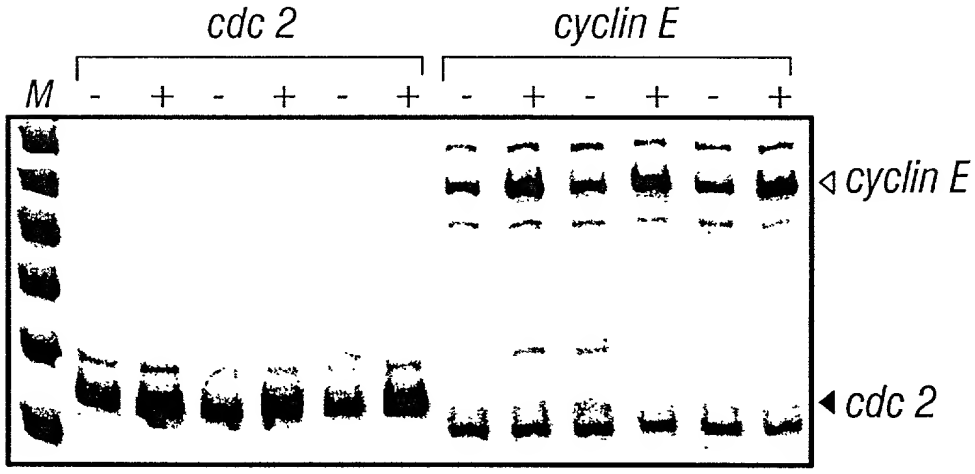

FIG. 16A

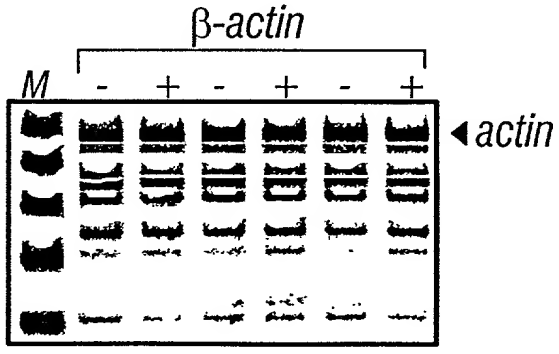

FIG. 16B

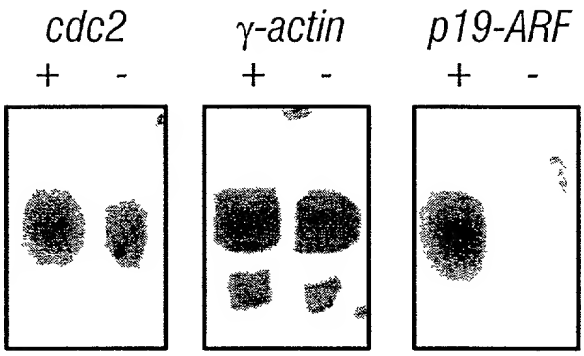

FIG. 17A

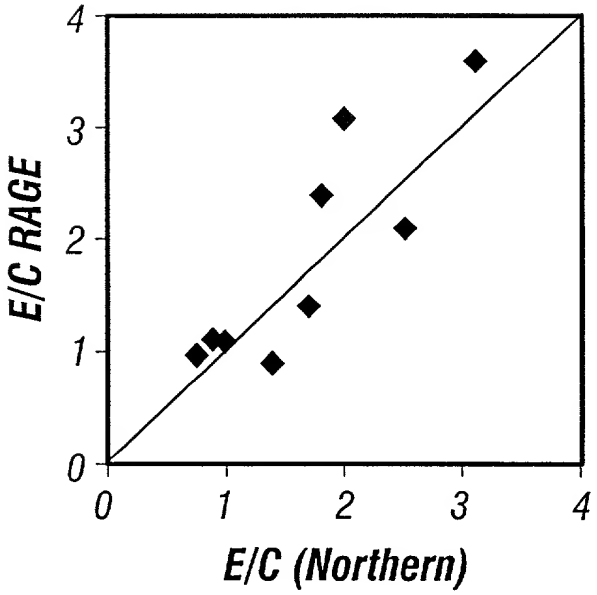

FIG. 17B

FIG. 18A

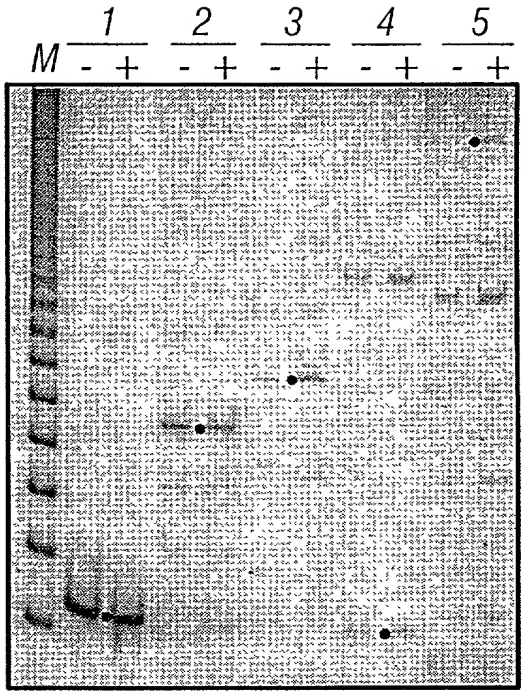

FIG. 18B

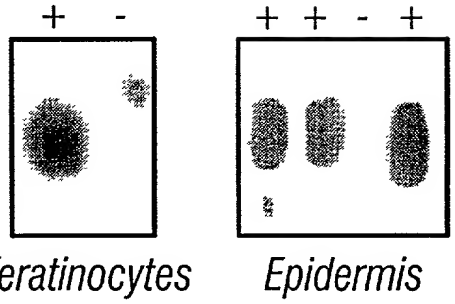

FIG. 18C

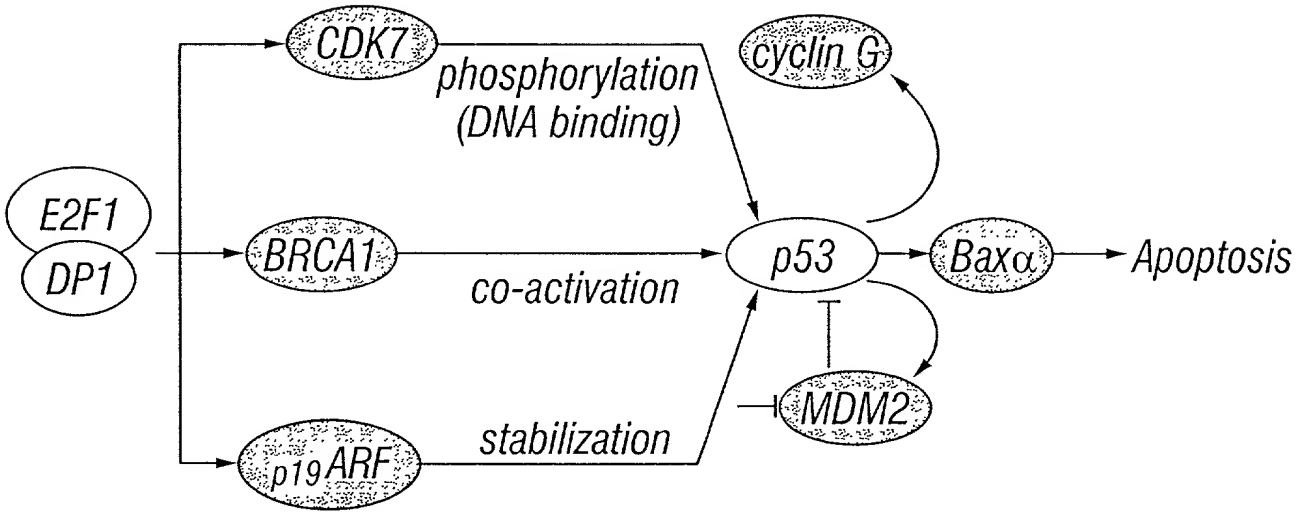
CDK7
phosphorylation
(DNA binding)
cyclin G
E2F1
DP1
BRCA1
co-activation
p53
Baxα
Apoptosis
MDM2
p19ARF
stabilization

FIG. 19

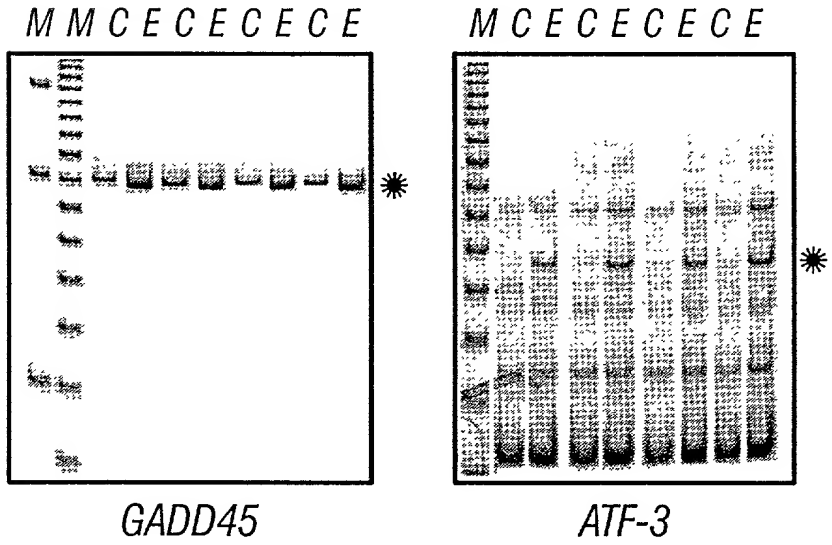
M M C E C E C E C E
M C E C E C E C E
GADD45
ATF-3

FIG. 20

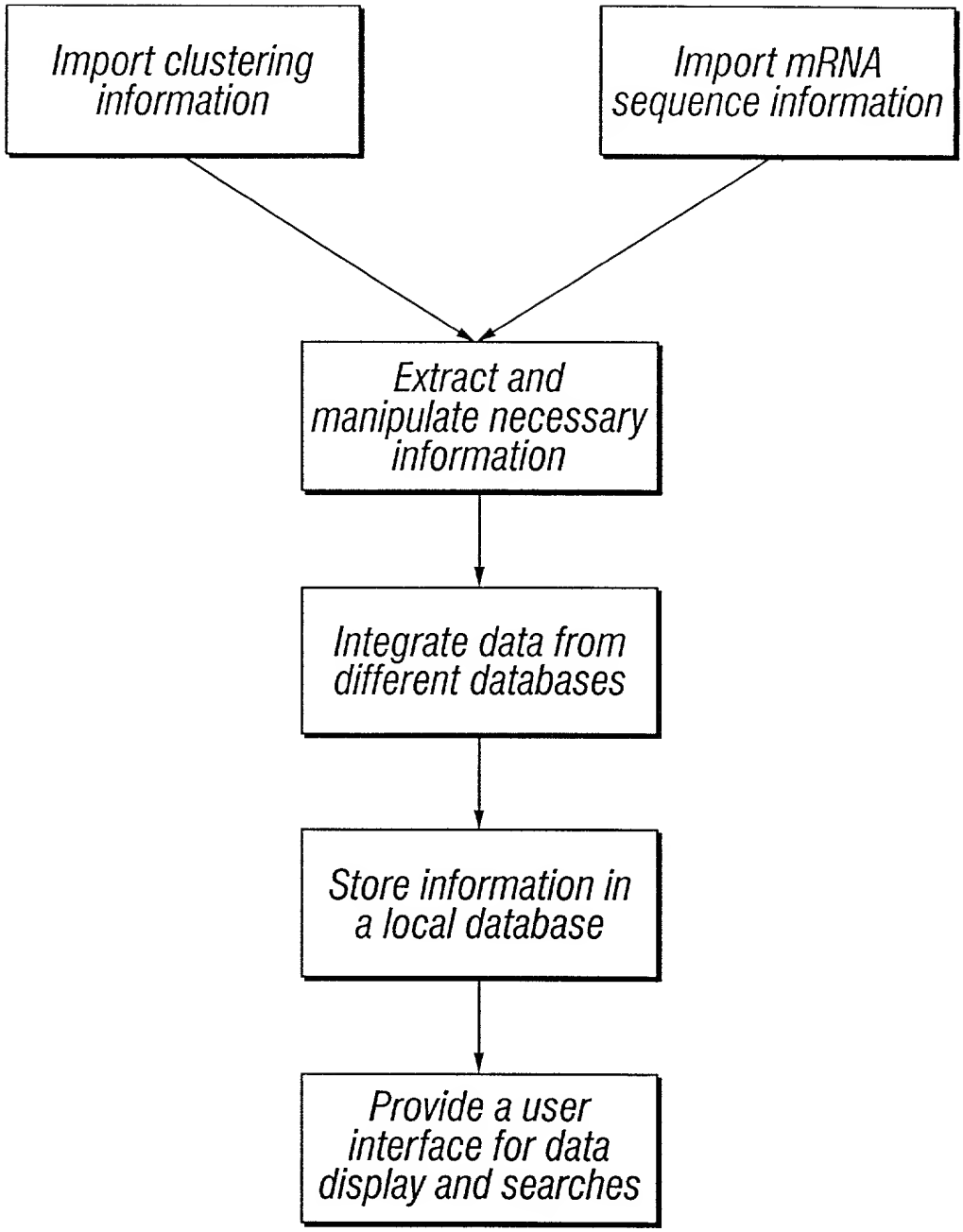
Import clustering information
Import mRNA sequence information
Extract and manipulate necessary information
Integrate data from different databases
Store information in a local database
Provide a user interface for data display and searches

FIG. 21